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INFLUENCE OF UNSATURATION ON FIBRINOLYTIC ACTIVITY OF FATTY ACIDS

Michael J. Surgalla
Earl D. Beesley
Robert R. Brubaker

MARCH 1967

DEPARTMENT OF THE ARMY
Fort Detrick
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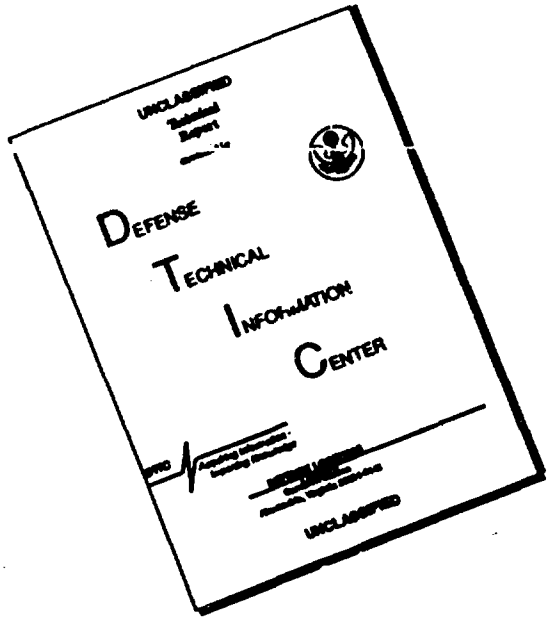

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INFLUENCE OF UNSATURATION ON FIBRINOLYTIC ACTIVITY OF FATTY ACIDS

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BIOLOGICAL SCIENCES LABORATORY

Project 1C014501B71A

March 1967

INFLUENCE OF UNSATURATION ON FIBRINOLYTIC ACTIVITY OF FATTY ACIDS

ABSTRACT

Long-chain saturated fatty acids are known to accelerate blood clotting and artificial thrombus formation in vitro, and to produce massive thrombosis and death in dogs and mice. We have found that some long-chain fatty acids induce fibrinolytic activity on bovine fibrin films, with some indication that unsaturated acids were most active. Twofold dilutions of the potassium salts of fatty acids were tested in 0.02-ml amounts for ability to lyse unheated bovine fibrin films. An attempt was made to establish the influence of unsaturation and chain length on fibrinolytic activity. The presence of one unsaturated bond appears to make little difference in activity of C16 fatty acids. In C18 and C20 acids, it increases activity roughly tenfold. In C22 and C24 acids it increases activity more than 100-fold. A possible role of fatty acids in regulation of fibrin formation and digestion is suggested.

Long-chain fatty acids have been reported to accelerate blood clotting and artificial thrombus formation in vitro[1,2] and to cause massive thrombosis and death in dogs.[3,4] Unsaturated fatty acids are relatively inactive. We have found recently that long-chain fatty acids can induce fibrinolysis on fibrin plates, and in this case it appeared that the unsaturated acids were more active than the saturated. These preliminary findings are confirmed and extended here.

Fibrinolysis was assayed on a modified Astrup plate prepared in the following manner. Fibrin films were prepared by dissolving Armour bovine fibrinogen (2.5 mg/ml) and Parke, Davis bovine thrombin, topical (50 NIH units/ml) in sodium borate buffer pH 7.7.[5] Fibrinogen solutions were sterilized by filtration and 10-ml volumes were added to 8.5-cm petri dishes. With the plates on a level surface, 0.5 ml of thrombin solution was added dropwise to the fibrinogen while the mixture was gently swirled to assure thorough distribution. Twofold dilutions of potassium salts of fatty acids were placed on fibrin films in 0.02-ml amounts. The lowest concentration that caused complete perforation of the fibrin film after incubation at 37 C for at least 4 hours was taken as the end point. Figure 1 shows the activity of twofold dilutions of sodium myristate. At concentrations of 20, 10, 5, 2.5, and 1.25 mM, 0.02-ml amounts placed on the fibrin film caused its complete perforation.

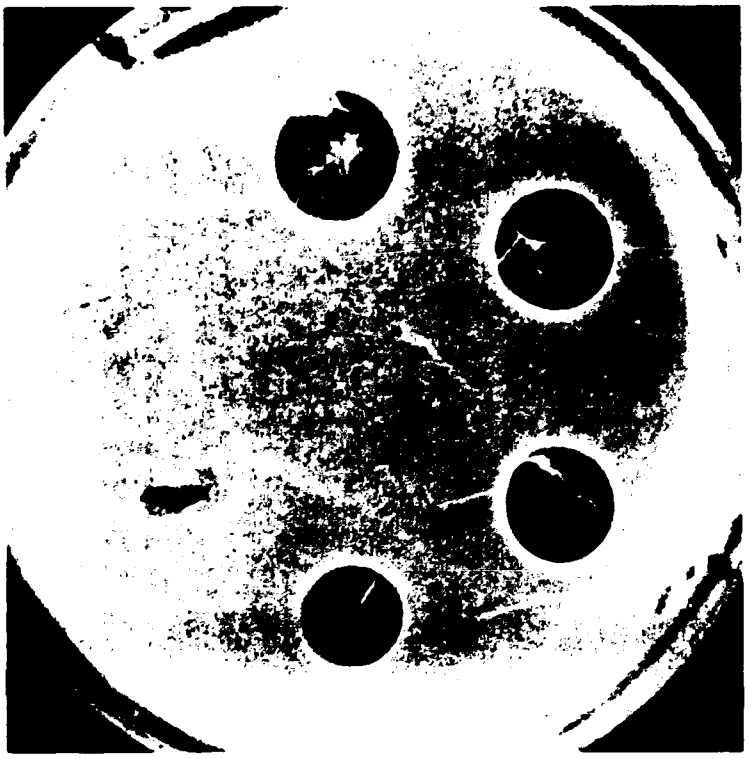

Figure 1. Activity of Sodium Myristate on Bovine Fibrin Film.

Saturated fatty acids having 16, 18, 20, 22, and 24 carbons were compared with acids with one unsaturated bond (Table 1).

TABLE 1. INFLUENCE OF UNSATURATION ON FIBRINOLYTIC ACTIVITY

		Active Concentration, mM Test Number				
		1	2	3	4	5
Palmitic	C16:0		5	0.3	0.3	
Palmitoleic	C16:1		0.5	0.3	0.3	
Stearic	C18:0		0.6	1	1	
Oleic	C18:1	0.2	0.06	0.2	0.08	
Elaidic	C18:1		0.06	0.2	0.2	
Arachidic	C20:0		1	5	1	
Eicosenoic	C20:1					0.08
Behenic	C22:0		>10	>10	>10	
Erucic	C22:1		0.03	0.02	0.04	
Lignoceric	C24:0					>10
Nervonic	C24:1					0.08

Palmitic and palmitoleic acids appear to be equally active. Presence of one unsaturated bond in C18 and C20 acids increases activity roughly tenfold. Presence of one unsaturated bond in C22 and C24 acids increases activity well over 100-fold.

The data in Table 2 suggest that although one unsaturated bond increases activity, polyunsaturation may decrease it. The fourth test of linoleic acid illustrates the frustrations encountered occasionally with the assay method.

TABLE 2. INFLUENCE OF POLYUNSATURATION

Fatty Acid		Active Concentration, mM Test Number 1	2	3	4	5
Oleic	C18:1	0.2	0.06	0.2	0.08	
Elaidic	C18:1		0.06	0.2	0.2	
Linoleic	C18:2	0.2	0.5	0.3	10	
Linolenic	C18:3		0.1	0.6	0.6	
Eicosenoic	C20:1					0.08
Arachidonic	C20:4			1	1	

Table 3 shows that saturated fatty acids of chain lengths 12 to 20 are fibrinolytic.

TABLE 3. INFLUENCE OF CHAIN LENGTH ON ACTIVITY OF SATURATED ACIDS

Fatty Acid		Active Concentration, mM Test Number 1	2	3	4
Lauric	C12	5	10	3	10
Myristic	C14	1	0.6	3	1
Pentadecanoic	C15		1	6	3
Palmitic	C16		5	0.3	0.3
Stearic	C18		0.6	1	1
Nonadecanoic	C19		6	3	1
Arachidic	C20		1	5	1
Behenic	C22		>10	>10	>10

Gans et al.[6] have suggested that plasminogen activator plays a role in preventing formation of thrombi because increased plasminogen activator occurs simultaneously with hypercoagulability following intravenous administration of endotoxin in dogs. Pig platelet aggregation induced by behenic acid is counteracted by linoleic or linolenic acids in vitro,[7] and elevated thrombotic tendency (platelet adhesion) in human patients is reduced by ingestion of linolenic acid.[8] Since fatty acids are non-fibrinolytic on fibrin films heated to destroy plasminogen, activation of plasminogen appears to be involved in fatty acid fibrinolytic activity. It appears, therefore, that attention should be given to the possible involvement of plasminogen activation in regulating early stages of thrombosis.

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13. ABSTRACT

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14. Key Words

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